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COLLAGENASE INHIBITORS IN 'PSEUDOMONAS' KERATITIS. ADJUNCTS TO ANTIBIOTIC THERAPY IN RABBITS

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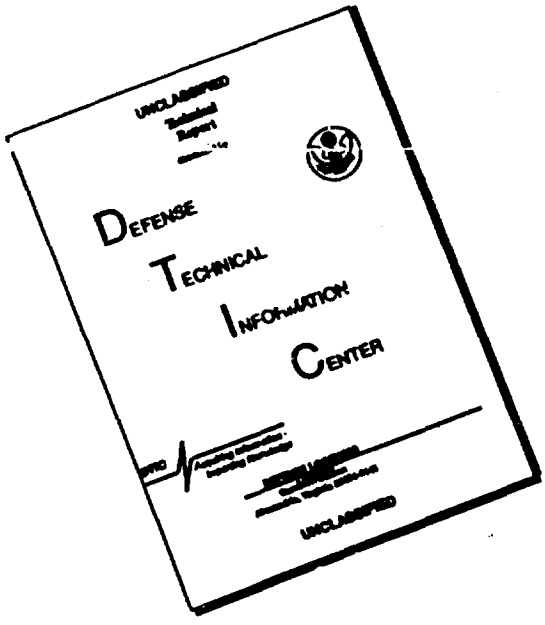

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13. ABSTRACT

Collagenase inhibitors as adjuncts to antibiotic therapy were evaluated in experimentally induced *Pseudomonas* keratitis in rabbits. Neither the combination of 1% edetic acid (EDTA) and 0.25% polymyxin B sulfate nor that of 1.2% cysteine and 0.25% polymyxin B sulfate was more effective than the polymyxin B sulfate alone. There was a small statistical difference in favor of a combination of edetic acid-cysteine-polymyxin B sulfate when it was compared with an edetic acid-polymyxin B sulfate combination. This difference, although statistically significant, did not appear to be clinically important in the treatment of our experimental model. (U)

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Collagenase Inhibitors in *Pseudomonas* Keratitis

Adjuncts to Antibiotic Therapy in Rabbits

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Collagenase inhibitors as adjuncts to antibiotic therapy were evaluated in experimentally induced *Pseudomonas* keratitis in rabbits. Neither the combination of 1% edetic acid (EDTA) and 0.25% polymyxin B sulfate nor that of 1.2% cysteine and 0.25% polymyxin B sulfate was more effective than the polymyxin B sulfate alone. There was a small statistical difference in favor of a combination of edetic acid-cysteine-polymyxin B sulfate when it was compared with an edetic acid-polymyxin B sulfate combination. This difference, although statistically significant, did not appear to be clinically important in the treatment of our experimental model.

In experimentally induced *Pseudomonas aeruginosa* keratitis, the organism produces a protease that is apparently collagenase.[1] Several investigators have reported recently that collagenase inhibitors, such as edetic acid (EDTA) and cysteine, are beneficial in experimental collagenase-induced keratitis.[2-4] The beneficial effect is mediated in part by chelation of the calcium necessary for activity of the enzyme collagenase.[5,6] In addition, edetic acid has a bactericidal effect on *P aeruginosa* by disorganizing the outer layer of the cell wall, which facilitates the penetration of antimicrobial agents.[7]

Edetic acid and antibiotic combinations have been shown to have a synergistic effect on *P aeruginosa* in vitro,[8] and Wilson has shown that edetic acid in vivo is an effective adjunct to antibiotic therapy in keratitis that has been experimentally induced with *Pseudomonas* extract.[9]

The purpose of this study was to determine whether or not collagenase inhibitors were beneficial adjuncts to the usual antibiotic treatment of an animal model of *Pseudomonas* corneal ulcer.

Materials and Methods

Drugs.—Sterile 1% edetic acid (2.9×10^{-2}M), pH 7.0, was obtained from the University of California Medical Center Pharmacy. The 1.2% L-cysteine-free base (10^{-1}M) and 0.25% polymyxin B sulfate were obtained from commercial manufacturers. All drugs were freshly prepared daily in sterile distilled water, and the pH of each solution was checked before and after use.

Strain of *Pseudomonas*.—The strain of *P aeruginosa* we used was obtained originally from a patient with an active corneal ulcer.[10] It was sensitive to 50-mg polymyxin B sulfate disks and was identified as type 6 (by the pyocine typing technique) at the Center for Disease Control, Atlanta. A 24-hour growth of this strain at 37 C on blood agar was diluted with physiological saline solution to produce a suspension standardized to 5% transmission at a wavelength of 540 nm against a clear blank in a spectrophotometer. This cell suspension contained approximately 10^9 viable organisms/ml.

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The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

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Experimental Model.—To produce *Pseudomonas* keratitis experimentally, we inoculated 45 female New Zealand white rabbits weighing approximately 2 kg each. Four days prior to inoculation, the eyes were anesthetized with one drop of proparacaine hydrochloride, and the lower punctum of each eye was cauterized for 20 seconds with a hot wire.[11] On the day of inoculation, the eyes were again anesthetized, and a circular area of central corneal epithelium, 5 mm in diameter, was removed with a platinum spatula. On the denuded surface, scratch marks were made in four directions with a five-pronged tattoo needle. Approximately 0.1 ml of the *Pseudomonas* suspension was applied to the corneas of both eyes, and the lids were sutured together with 4-0 black silk and left sutured for 48 hours.

Within 48 hours, all 90 eyes had developed marked purulent conjunctivitis, mild overall corneal haze, and a whitish ulcer infiltrate centrally that usually occupied 25% to 50% of the corneal area (Fig 1). This technique had been used previously with similar results.[11]

Experimental Design.—The right eyes of the infected rabbits were treated with polymyxin B sulfate, alone and in various combinations with the collagenase inhibitors edetic acid and cysteine; the left eyes served as controls, saline solution being used in place of one or more of the drugs. Treatment was begun 48 hours after the *Pseudomonas* inoculation and was continued for five days. The reason for withholding medication for 48 hours was to create a situation that would resemble an actual clinical situation, since this would enhance the importance of any observed therapeutic benefit.

The infected animals were randomly divided into four groups of ten rabbits each and one group of five rabbits. In group 1, polymyxin B sulfate alone was compared with saline solution; in group 2, a combination of polymyxin B sulfate and edetic acid was compared with that of polymyxin B sulfate and saline solution; in group 3, a combination of polymyxin B sulfate and cysteine was compared with that of polymyxin B sulfate and saline solution; and in group 4 a combination of polymyxin B sulfate, edetic acid, and cysteine was compared with that of polymyxin B sulfate, edetic acid, and saline solution (Table 1).

An amount of each medication, 0.12 ml (2 drops), was applied every two hours during the day (7 AM to 6 PM), with five-minute intervals between drops of different drugs when more than one was applied. In addition, 0.1 ml of each solution was given subconjunctivally in different quadrants of the

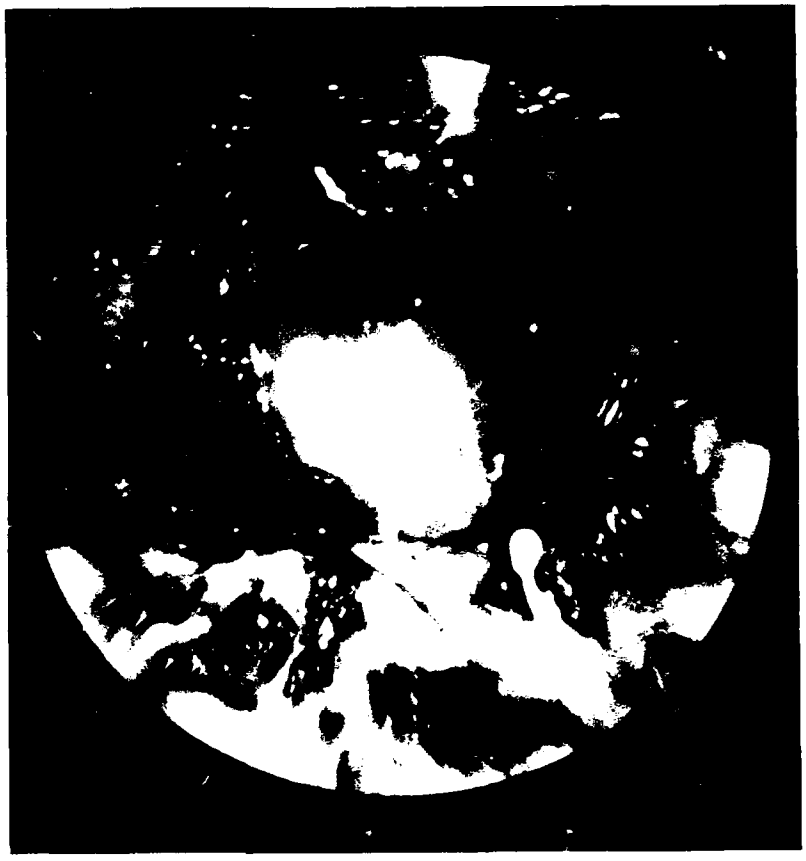


Fig 1.—Representative corneal ulcer infiltrate before therapy at 48 hours. Infiltrate graded +2 (area) and +2 (density). Final score of severity, +4.

Table 1.—Treatment Groups

Group	Right Eye	Left Eye
1	Polymyxin B sulfate	Saline solution
2	Edetic acid & polymyxin B sulfate	Saline solution & polymyxin B sulfate
3	Cysteine & polymyxin B sulfate	Saline solution & polymyxin B sulfate
4	Cysteine, edetic acid, polymyxin B sulfate	Saline, edetic acid, polymyxin B sulfate

Table 2.—Method of Scoring Severity of Experimental *Pseudomonas* Keratitis*

Area of Infiltrate (% of Corneal Surface Affected)	Grade	Density of Infiltrate	Grade
0	0	None	0
0-25	+1	Faint	+1
25-50	+2	Moderate	+2
50-75	+3	Severe	+3
75-100	+4	Very severe (chalky white)	+4

* Area grade and density grade totaled to express severity score for each eye. Maximum possible score is +8.

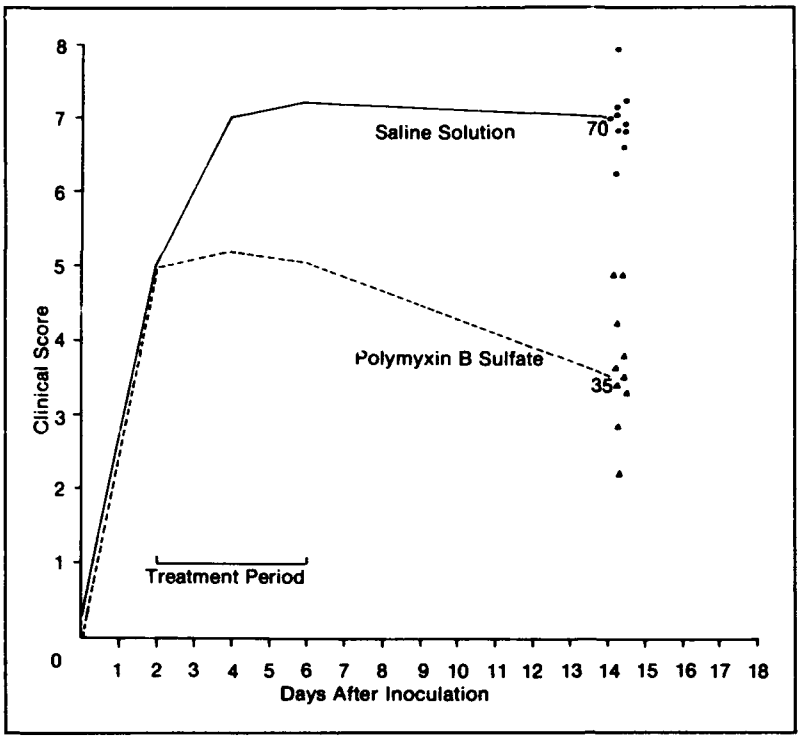


Fig 2.—Right eyes treated with polymyxin B sulfate (triangles-dashed line) had substantially less keratitis than left eyes treated with saline solution (dots-solid line).

eye once a night.

The special group of five infected animals received 0.1 ml of each solution intravenously via the marginal ear vein daily for five days. The reason for this additional control was to verify that subconjunctival therapy received in the right eye could not cross over systemically to affect the control eye.

All treatments were administered by an investigator who was not informed of the content of the solution he was injecting.

Scoring of the Corneal Lesions.—The animals were examined grossly with a slit-lamp every day for two weeks, representative photographs were taken, and the lesions were graded by two observers who did not know which form of treatment the animals had received. The area and the density of the corneal ulcer infiltrates were graded separately from 0 to +4. The area grade and the density grade were then added together to give the score of the severity of the keratitis in each eye (Table 2).

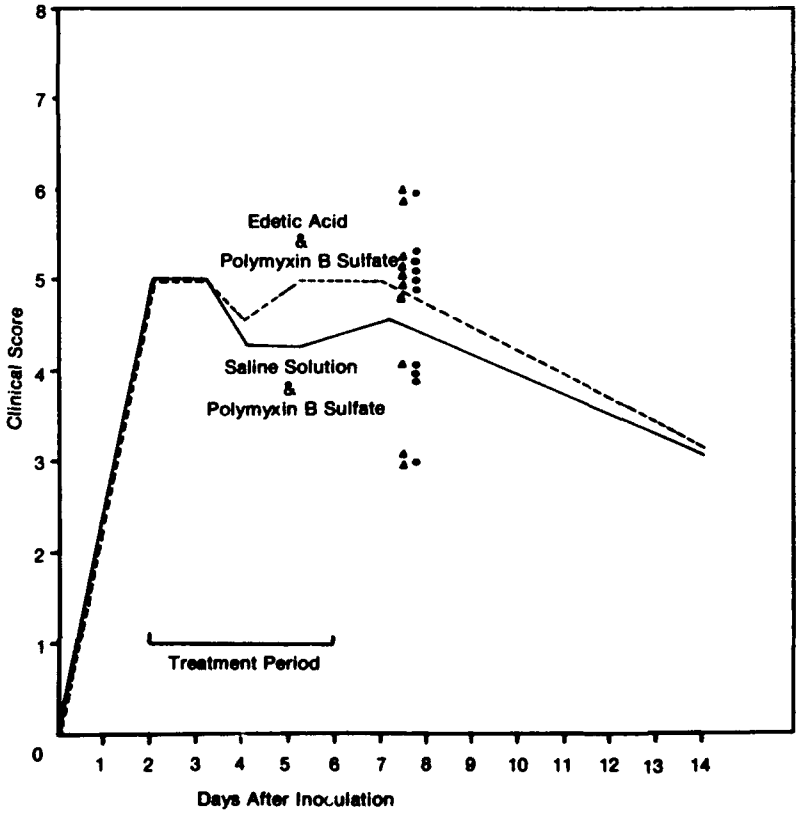
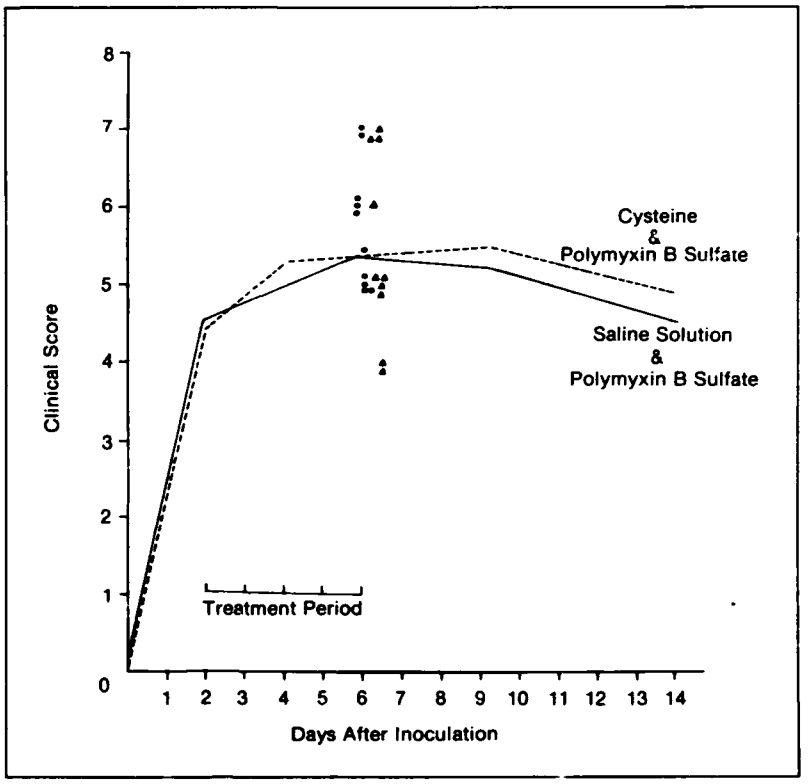


Fig 3.—Right eyes treated with edetic acid and polymyxin B sulfate (triangles-dashed line) had nearly same degree of keratitis as left eyes treated with saline solution and polymyxin B sulfate (dots-solid line).

Results

All of the medications were well tolerated. None of the rabbits died during the study. The results in each group were plotted on a series of scattergrams to show a progression of the ulcer infiltrates (Fig 2 to 6). In group 1 (Fig 2), there was a statistically significant difference between the polymyxin B sulfate-treated right eyes and the saline solution-treated left eyes. No statistically significant differences were found in groups 2 and 3 (Fig 3 and 4) on postinoculation days 7 or 14. In group 4 (Fig 5), the cysteine-edetic acid-polymyxin B sulfate combination in comparison with the saline solution-edetic acid-polymyxin B sulfate combination resulted in a statistically significant difference in favor of the cysteine-edetic acid-polymyxin B sulfate combination ($P = .01$ by χ^2 for small frequencies when the

Fig 4.—Right eyes treated with cysteine and polymyxin B sulfate (triangle-dashed line) had nearly same degree of keratitis as left eyes treated with saline solution and polymyxin B sulfate (dots-solid line).



clinical score was less than two at day 14).[12] If the χ^2 was applied when the clinical score was equal to or more than 2, then the differences were no longer statistically significant.

Intravenous medication had no beneficial effect on the natural course of the disease; the keratitis induced in the rabbits treated intravenously with the various drugs followed the same course as that in the controls that received saline solutions.

All of the therapeutic combinations tested are compared in Fig 6.

Comment

In this experimental model, no benefit was derived from the addition of either 1% edetic acid or 1.2% cysteine to 0.25% polymyxin B sulfate when the effects were compared with the effect of polymyxin B sulfate alone. When both of the collagenase inhibitors were added, there was a small difference between this three-way combination (edetic acid, cysteine, and polymyxin B sulfate) and the two-way combination (edetic acid and polymyxin B sulfate). This difference, although "statistically" significant, did not appear to offer any practical "clinical" advantage. All of the observers agreed that the differences were not clinically important. The three-part combination might, of course, have a more favorable effect in vivo if treatment were begun earlier and administered longer. However, our study was expressly designed to show which of these drug combinations, if any, would be more effective than polymyxin B sulfate alone late in the experimental disease. It was assumed that the experimental disease at this stage would more closely correspond to the human disease, the treatment of which is so

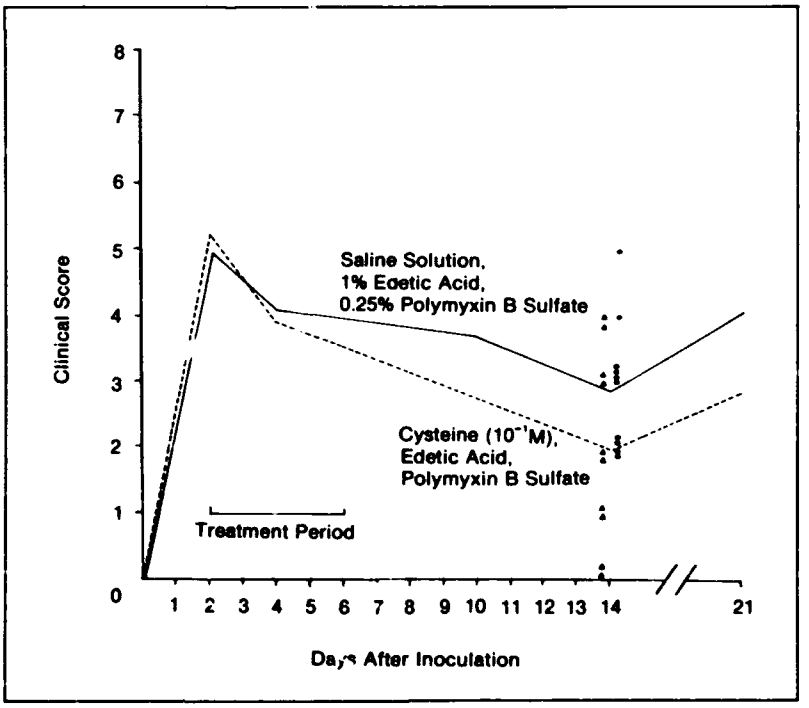


Fig 5.—Right eyes treated with cysteine, edetic acid, and polymyxin B sulfate (triangles-dashed line) had substantially less keratitis at day 14 than left eyes treated with saline solution, edetic acid, and polymyxin B sulfate (dots-solid line).

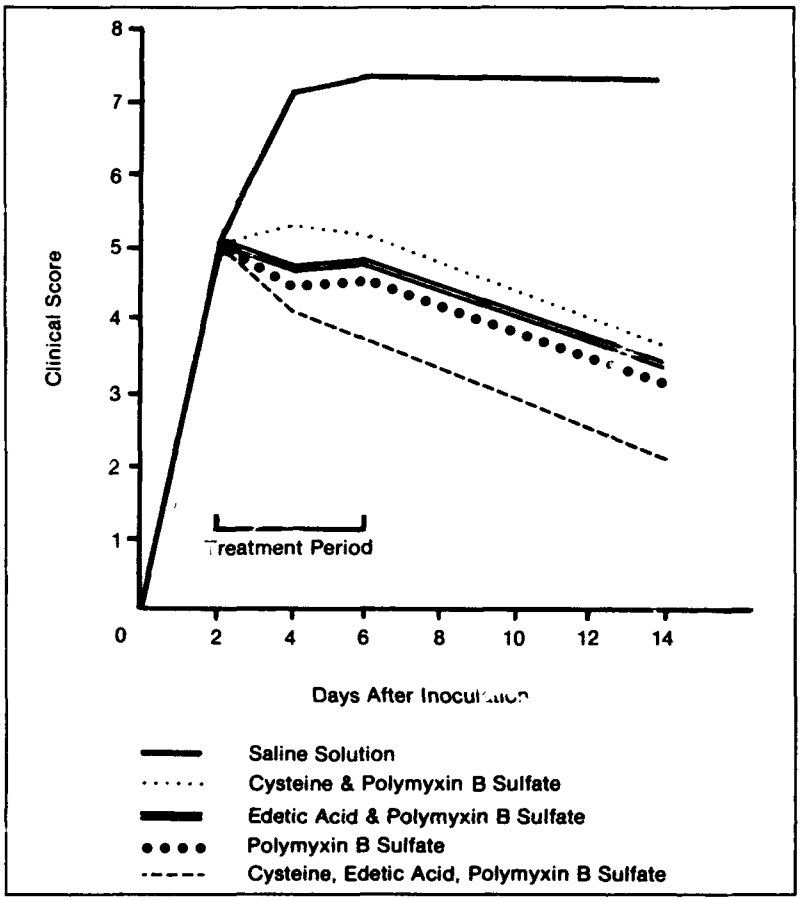


Fig 6.—Comparison of effects of treating experimentally induced *Pseudomonas* keratitis with polymyxin B sulfate alone, with polymyxin B sulfate and edetic acid or cysteine, with polymyxin B sulfate combined with edetic acid and cysteine, and with saline solution alone.

frequently delayed.

The low concentration of polymyxin B sulfate was chosen so that any therapeutic effect displayed by the collagenase inhibitors would not be masked; the antibiotic even in this strength was effective. E. Chowchuveck, MD; M. Okumoto, MA; and C. Yoneda, MD, using this strain of *P aeruginosa* and this model, have found 1% edetic acid to have a slightly beneficial effect compared with saline solution (unpublished data). The use of higher concentrations of these collagenase inhibitors has recently been recommended.[4]

The clinical picture of the disease process in rabbits and humans is slightly different. Experimentally induced *Pseudomonas* infections of the cornea in the rabbit are mainly a necrosis of the epithelium and stroma and hence an ulcer infiltrate. In human cases, a similar sequence can be seen, but frequently this can appear more as a corneal melting process. This process may be different than stromal disease seen in the rabbit model; thus, the effects of collagenase inhibitors on corneal melting in humans could not be determined by the present study. The therapeutic effect of collagenase inhibitors on human cases of *Pseudomonas* corneal ulcer will depend on the results of well-controlled studies in the future. In our animal model, topically and subconjunctivally administered edetic acid and cysteine combinations with polymyxin B sulfate did not have a more beneficial effect than polymyxin B sulfate alone.

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The experiments reported herein were conducted according to the principles given in *Guide for Laboratory Animal Facilities and Care* prepared by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences–National Research Council.

Key Words.—*Pseudomonas aeruginosa;* collagenase inhibitors; keratitis; corneal ulcer; antibiotics; polymyxin B sulfate; cysteine; edetic acid (EDTA); subconjunctival antibiotics; cornea.

Nonproprietary Names and Trademarks of Drugs

Proparacaine hydrochloride—*Alcaine, Ophthaine.*

Edetic acid—*Nullapons, Sequestrene, Versene Acid.*

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